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(54) Title: DETECTION OF FERMENTATION–RELATED MICROORGANISMS

(57) Abstract

Unique DNA sequences are provided which are useful in identifying different fermentation–related microorganisms, such as those involved in fermentations. These unique DNA sequences can be used to provide oligonucleotide primers in PCR based analysis for the identification of fermentation–related microorganisms. The DNA sequences of the present invention include the internal transcribed spacer (ITS) of the ribosomal RNA gene regions of particular fermentation–related microorganisms, as well as oligonucleotide primers which are derived from these regions which are capable of identifying the particular microorganism.

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DETECTION OF FERMENTATION-RELATED MICROORGANISMS

Field of the Invention

The present invention relates to assays to detect fermentation-related microorganisms.

Description of the Related Art

In traditional wine-making, the indigenous yeasts ferment the grape must. Most modern wine-makers, however, inoculate with a pure culture of a selected yeast strain to ensure a rapid, reliable and predictable fermentation. It is thought that indigenous yeasts are suppressed by the competitive effect of addition of a high-density monoculture, but some evidence suggests that indigenous yeast can still participate in the fermentation. A range of commercial yeasts with different wine-making characteristics is available, and a number of those yeast strains may be used in a single winery. Furthermore, unwanted microorganisms may be present which lead to spoilage. Therefore, there is a need for a rapid, simple and accurate method for identifying microorganisms in starter cultures and fermentations.

Past techniques for detecting and identifying fermentation-related microorganisms, especially yeast, include colony morphology, fermentation performance, sugar fermentation tests, tolerance to various stresses (e.g., ethanol tolerance in brewing), phenotypes with functional relevance (e.g., flocculation in brewing), nutritional requirements (e.g., oxygen), and resistance and sensitivity levels of cycloheximide. These methods, however, have numerous disadvantages, including lengthy analysis periods, inability to differentiate, e.g., different strains of yeast, and lack of reproducibility.

Recent developments in molecular biology and protein chemistry have provided new methods for identifying microorganisms, including DNA restriction fragment length polymorphisms, protein electrophoresis patterns and chromosome fingerprinting. Such techniques have been used for identifying fermentation-related microorganisms. See, for example, Casey et al, *Journal of the American Society of Brewing Chemists*, 48(3):100-106, 1990; Degre et al, *American Journal of Enology and Viticulture*, 40(4):309-315, 1989; Guillamon et al, *Systematic and Applied Microbiology*, 19:122-132, 1992; Hoeben et al, *Current Genetics*, 10:371-379, 1986; Mozina et al, *Letters in Applied Microbiology*, 24(4):311-315, 1997; Paffetti et al, *Research Microbiology*, 146:587-594, 1995; Panchal et al, *Journal of the Institute of Brewing*, 93:325-327, 1987; Querol et al, *Systematic and Applied Microbiology*, 15:439-446, 1992; Vezinhet et al, *Applied Microbiology and Biotechnology*, 32:568-571, 1990; and Vezinhet et al, *American Journal of Enology and Viticulture*, 43(1):83-86, 1992.

Polymerase chain reaction (PCR)-based techniques have also been used to detect fermentation-related microorganisms. See, for example, DeBarros Lopes et al, *Applied and Environmental Microbiology*, 62(12):4514-4520, 1996; Fell, *Molecular Marine Biology and Biotechnology*, 2(3):174-180, 1993; Fell, *Journal of Industrial Microbiology*, 14(6):475-477, 1995; Ibeas et al, *Applied and Environmental Microbiology*, 62(3):998-1003, 1996; Lavallee et al, *American Journal of Enology and Viticulture*, 45(1):86-91, 1994; Lieckfeldt et al, *Journal of Basic Microbiology*, 33(6):413-425, 1993; and Ness et al, *J. Sci. Food Agric.*, 62:89-94, 1993.

Ribosomal genes are suitable for use as molecular probe targets because of their high copy number. Non-transcribed and transcribed spacer sequences associated with ribosomal genes are usually poorly conserved and, thus, are

advantageously used as target sequences for the detection of recent evolutionary divergence. Fungal rRNA genes are organized in units. Each unit encodes mature subunits of 18S, 5.8S, and 28S rRNA. The internal transcribed spacer (ITS) region lies between the 18S and 28S rRNA genes and contains two variable non-coding spacers (referred to as ITS1 and ITS2) and the 5.8S rRNA gene (White et al., 1990; In: *PCR Protocols*; Eds.: Innes et al.; pages 315-322). In addition, the transcriptional units are separated by non-transcribed spacer sequences (NTSs). The ITS and NTS sequences are particularly suitable for the detection of different fungal pathogens.

Kumeda et al (*Applied and Environmental Microbiology*, 62(8):2947-2952, 1996) describes use of PCR to amplify ribosomal DNA internal transcribed spacers in order to differentiate species of *Aspergillus* Section *Flavi*. The ITS1-5.8S-ITS2 region was amplified using universal primers, and the PCR product analyzed by the principle of single-strand conformation polymorphism. In addition, Gardes et al (In: *Methods in Molecular Biology, Vol. 50:Species Diagnostics Protocols: PCR and Other Nucleic Acid Methods*, Ed. J.P. Clapp, Humana Press, Totowa, NJ, (1996) pp. 177-186) describes restriction fragment length polymorphism (RFLP) analysis of fungal ITS regions amplified by PCR.

The PCR amplification of fungal ITS has also been described using other than universal primers. These methods allow for more specificity in identifying classes of fungi, or particular species of fungi. Thus, Gardes and Bruns (*Molecular Ecology*, 2:113-118, 1993) identified ITS primers which allow differentiation of DNA from basidiomycetes against ascomycete DNA. Identification of specific species has been observed using PCR primers directed to unique sequences in the ITS1 and/or ITS2 regions of fungal pathogens. See, for example, Hamelin et al, *Applied and Environmental Microbiology*, 62(11):4026-4031, 1996; Mazzola et al, *Phytopathology*, 86(4):354-360, 1996;

O'Gorman et al, *Canadian Journal of Botany*, 72:342-346, 1994; and U.S. Patent No. 5,585,238 to Ligon et al.

The present invention addresses the problem of detecting and identifying fermentation-related microorganisms by PCR-based techniques.

Summary of the Invention

The present invention is directed to the identification of different fermentation-related microorganisms, particularly those involved in the production of wine. The present invention provides DNA sequences which exhibit variability between different fermentation-related microorganisms. In particular, the present invention identifies regions of DNA sequence located in the internal transcribed spacer (ITS) of the ribosomal RNA gene regions of various fermentation-related microorganisms. Primers derived from the ITS can be used in polymerase chain reaction (PCR) based diagnostic assays to determine the presence or absence of specific fermentation-related microorganisms, including those involved in the production of wine. The primers can also be used as molecular probes to detect the presence of target DNA.

Thus, in one aspect, the present invention provides an isolated double stranded nucleic acid of the full length ITS1 or ITS2 region of a fermentation-related microorganism. More particularly, the DNA sequence is selected from among Sequence ID NOS: 13 to 36.

In another aspect, the present invention provides an oligonucleotide primer for identification of a fermentation-related microorganism, wherein the primer is a divergent portion of the ITS1 or ITS2 region of a fermentation-related microorganism. More particularly, the oligonucleotide primer is selected from among Sequence ID NOS: 65 to 98. Furthermore, the oligonucleotide primers may be selected from among sequences which contain at least 10 contiguous nucleotide bases from one of SEQ ID NOS: 65 to 98,

primers which contain at least 10 contiguous nucleotide bases from one of SEQ ID NOS: 65 to 98 contiguous with 1 to 15 nucleotide bases in the 5' and/or 3' direction of corresponding SEQ ID NOS: 37 to 64, and primers of 10 nucleotide bases or longer which contain at least 5 contiguous nucleotide bases from one of SEQ ID NOS: 65 to 98 contiguous with from 1 to 15 nucleotide bases in the 5' and/or 3' direction of corresponding SEQ ID NOS: 37 to 64. A pair of the foregoing oligonucleotide primers for use in the amplification-based detection of an ITS of a fermentation-related microorganism is also provided.

In yet another aspect, a method is provided for detection of a fermentation-related microorganism which comprises: (a) obtaining DNA from a fungal culture or colony isolated from a fermentation, or from an organism present in a fermentation beverage; (b) amplifying a part of the ITS of the fermentation-related microorganism using the DNA as a template in a polymerase chain reaction with the aforementioned oligonucleotide primers; and (c) visualizing the amplified part of the ITS sequence to determine whether the fermentation-related microorganism is present.

In still another aspect, kits are provided which are useful in detecting fermentation-related microorganisms.

Brief Description of the Sequences in the Sequence Listing:

SEQ ID NO: 1	DNA sequence for the internal transcribed spacer of *Saccharomyces cerevisiae* and *Saccharomyces bayanus*.
SEQ ID NO: 2	DNA sequence for the internal transcribed spacer of *Saccharomycodes ludwigii*.
SEQ ID NO: 3	DNA sequence for the internal transcribed spacer of *Dekkera bruxellensis*.
SEQ ID NO: 4	DNA sequence for the internal transcribed spacer of *Dekkera intermedia*.

SEQ ID NO: 5 DNA sequence for the internal transcribed spacer of *Botrytis cinerea*.

SEQ ID NO: 6 DNA sequence for the internal transcribed spacer of *Penicillium crustosum*.

SEQ ID NO: 7 DNA sequence for the internal transcribed spacer of *Penicillium expansum*.

SEQ ID NO: 8 DNA sequence for the internal transcribed spacer of *Hanseniaspora guilliermondii*.

SEQ ID NO: 9 DNA sequence for the internal transcribed spacer of *Debaryomyces carsonii*.

SEQ ID NO: 10 DNA sequence for the internal transcribed spacer of *Pichia anomala*.

SEQ ID NO: 11 DNA sequence for the internal transcribed spacer of *Pichia kluyveri*.

SEQ ID NO: 12 DNA sequence for the internal transcribed spacer of *Candida krusei*.

SEQ ID NO: 13 DNA sequence for the ITS1 of *Saccharomyces cerevisiae* and *Saccharomyces bayanus*.

SEQ ID NO: 14 DNA sequence for the ITS2 of *Saccharomyces cerevisiae* and *Saccharomyces bayanus*.

SEQ ID NO: 15 DNA sequence for the ITS1 of *Saccharomycodes ludwigii*.

SEQ ID NO: 16 DNA sequence for the ITS2 of *Saccharomycodes ludwigii*.

SEQ ID NO: 17 DNA sequence for the ITS1 of *Dekkera bruxellensis*.

SEQ ID NO: 18 DNA sequence for the ITS2 of *Dekkera bruxellensis*.

SEQ ID NO: 19 DNA sequence for the ITS1 of *Dekkera intermedia*.

SEQ ID NO: 20 DNA sequence for the ITS2 of *Dekkera intermedia*.

SEQ ID NO: 21 DNA sequence for the ITS1 of *Botrytis cinerea*.

SEQ ID NO: 22 DNA sequence for the ITS2 of *Botrytis cinerea*.

SEQ ID NO: 23 DNA sequence for the ITS1 of *Penicillium crustosum*.

SEQ ID NO: 24 DNA sequence for the ITS2 of *Penicillium crustosum*.

SEQ ID NO: 25 DNA sequence for the ITS1 of *Penicillium expansum*.

SEQ ID NO: 26 DNA sequence for the ITS2 of *Penicillium expansum*.

SEQ ID NO: 27 DNA sequence for the ITS1 of *Hanseniaspora guilliermondii*.

SEQ ID NO: 28 DNA sequence for the ITS2 of *Hanseniaspora guilliermondii*.

SEQ ID NO: 29 DNA sequence for the ITS1 of *Debaryomyces carsonii*.

SEQ ID NO: 30 DNA sequence for the ITS2 of *Debaryomyces carsonii*.

SEQ ID NO: 31 DNA sequence for the ITS1 of *Pichia anomala*.

SEQ ID NO: 32 DNA sequence for the ITS2 of *Pichia anomala*.

SEQ ID NO: 33 DNA sequence for the ITS1 of *Pichia kluyveri*.

SEQ ID NO: 34 DNA sequence for the ITS2 of *Pichia kluyveri*.

SEQ ID NO: 35 DNA sequence for the ITS1 of *Candida krusei*.

SEQ ID NO: 36 DNA sequence for the ITS2 of *Candida krusei*.

SEQ ID NO: 37 Oligonucleotide Sequence SXUITS1a.

SEQ ID NO: 38 Oligonucleotide Sequence SXLITS2a.

SEQ ID NO: 39 Oligonucleotide Sequence SXUITS1b.

SEQ ID NO: 40 Oligonucleotide Sequence SXLITS2b.

SEQ ID NO: 41 Oligonucleotide Sequence SLUITS1a.

SEQ ID NO: 42 Oligonucleotide Sequence SLLITS2a.

SEQ ID NO: 43 Oligonucleotide Sequence SLUITS1b.

SEQ ID NO: 44 Oligonucleotide Sequence SLLITS2b.

SEQ ID NO: 45 Oligonucleotide Sequence BRUITS1a.

SEQ ID NO: 46 Oligonucleotide Sequence BRLITS2.

SEQ ID NO: 47 Oligonucleotide Sequence BRUITS1b.

SEQ ID NO: 48 Oligonucleotide Sequence BCUITS1a.

SEQ ID NO: 49 Oligonucleotide Sequence BCLITS2.
SEQ ID NO: 50 Oligonucleotide Sequence BCUITS1b.
SEQ ID NO: 51 Oligonucleotide Sequence PXUITS1a.
SEQ ID NO: 52 Oligonucleotide Sequence PXLITS2a.
SEQ ID NO: 53 Oligonucleotide Sequence PXUITS1b.
SEQ ID NO: 54 Oligonucleotide Sequence PXLITS2b.
SEQ ID NO: 55 Oligonucleotide Sequence HGUITS1.
SEQ ID NO: 56 Oligonucleotide Sequence HGLITS2.
SEQ ID NO: 57 Oligonucleotide Sequence DXLITS2.
SEQ ID NO: 58 Oligonucleotide Sequence PAUITS1.
SEQ ID NO: 59 Oligonucleotide Sequence PALITS2.
SEQ ID NO: 60 Oligonucleotide Sequence PKLITS2.
SEQ ID NO: 61 Oligonucleotide Sequence CKUITS1.
SEQ ID NO: 62 Oligonucleotide Sequence CKLITS2.
SEQ ID NO: 63 Oligonucleotide Sequence SXU99.
SEQ ID NO: 64 Oligonucleotide Sequence SXL658.
SEQ ID NO: 65 Oligonucleotide Sequence SXU102.
SEQ ID NO: 66 Oligonucleotide Sequence SXL661.
SEQ ID NO: 67 Oligonucleotide Sequence SLU85.
SEQ ID NO: 68 Oligonucleotide Sequence SLL635.
SEQ ID NO: 69 Oligonucleotide Sequence SLU88.
SEQ ID NO: 70 Oligonucleotide Sequence SLL636.
SEQ ID NO: 71 Oligonucleotide Sequence SLU136.
SEQ ID NO: 72 Oligonucleotide Sequence SLL634.
SEQ ID NO: 73 Oligonucleotide Sequence BRU53A.
SEQ ID NO: 74 Oligonucleotide Sequence BRU53B.
SEQ ID NO: 75 Oligonucleotide Sequence BRU77.
SEQ ID NO: 76 Oligonucleotide Sequence BRL339.
SEQ ID NO: 77 Oligonucleotide Sequence BRL367.

SEQ ID NO: 78 Oligonucleotide Sequence BRL390.
SEQ ID NO: 79 Oligonucleotide Sequence BCU136.
SEQ ID NO: 80 Oligonucleotide Sequence BCL393.
SEQ ID NO: 81 Oligonucleotide Sequence BCU142.
SEQ ID NO: 82 Oligonucleotide Sequence PXU87.
SEQ ID NO: 83 Oligonucleotide Sequence PXL495.
SEQ ID NO: 84 Oligonucleotide Sequence PXU86.
SEQ ID NO: 85 Oligonucleotide Sequence PXL482.
SEQ ID NO: 86 Oligonucleotide Sequence PXL491.
SEQ ID NO: 87 Oligonucleotide Sequence HGU193.
SEQ ID NO: 88 Oligonucleotide Sequence HGU231.
SEQ ID NO: 89 Oligonucleotide Sequence HGL601.
SEQ ID NO: 90 Oligonucleotide Sequence DXL447.
SEQ ID NO: 91 Oligonucleotide Sequence DXL526.
SEQ ID NO: 92 Oligonucleotide Sequence PAU133.
SEQ ID NO: 93 Oligonucleotide Sequence PAL451.
SEQ ID NO: 94 Oligonucleotide Sequence PKL356.
SEQ ID NO: 95 Oligonucleotide Sequence CKU104.
SEQ ID NO: 96 Oligonucleotide Sequence CKL354.
SEQ ID NO: 97 Oligonucleotide Sequence ITS5.
SEQ ID NO: 98 Oligonucleotide Sequence ITS4.

Detailed Description Of The Invention

The present invention provides unique DNA sequences which are useful in identifying fermentation-related microorganisms. These unique DNA sequences can be used as primers in PCR-based analysis for the identification of fermentation-related microorganisms, or as molecular probes to detect the presence of DNA from fermentation-related microorganisms. The DNA sequences of the present invention include the internal transcribed spacer (ITS)

of the ribosomal RNA gene regions of specific fermentation-related microorganisms, as well as primers that are derived from these regions which are capable of identifying the particular microorganism.

The DNA sequences of the invention are from the ITS of the ribosomal RNA gene region of fermentation-related microorganisms. However, the present invention is not limited to detecting the presence of the microorganisms in fermentation operations, *i.e.*, the invention can be used to detect the presence of such microorganisms from any source. There is variability in the ITS DNA sequences from different microorganisms. The ITS sequences can be aligned and compared. Primers can be designed based on regions within the ITS regions that contain the greatest differences in sequence among the fermentation-related microorganisms. The sequences and primers based on these sequences can be used to identify specific microorganisms.

DNA sequences of particular interest include ITS DNA sequences from *Saccharomyces* sp., especially *Saccharomyces cerevisiae* and *Saccharomyces bayanus*; *Saccharomycodes* sp., especially *Saccharomycodes ludwigii*; *Dekkera* sp., especially *Dekkera bruxellensis* and *Dekkera intermedia*; *Botrytis* sp., especially *Botrytis cinerea*; *Penicillium* sp., especially *Penicillium crustosum* and *Penicillium expansum*; *Hanseniaspora* sp., especially *Hanseniaspora guilliermondii*; *Debaryomyces* sp., especially *Debaryomyces carsonii*; *Pichia* sp., especially *Pichia anomala* and *Pichia kluyveri*; and *Candida* sp., especially *Candida krusei*. The ITS DNA sequences, as well as primers of interest, are set forth in SEQUENCE ID NOS: 1-100. The sequences are useful in PCR-based identification of fermentation-related microorganisms.

Methods for use of the primer sequences of the invention in PCR analysis are well known in the art. For example, see U.S. Patent Nos. 4,683,195; 4,683,202 and 5,585,238, the contents of all of which are hereby incorporated by reference.

The primer sequences of the invention can also be used as molecular probes to detect the presence of target DNA. The Tm for the primers ranges from about 48-58° C at 50 mM salt. The hybridization temperature is approximately 5-10° C below the melting temperature. Thus, the primers are hybridized to target DNA typically at a temperature ranging from about 43-55° C. Final wash conditions generally range from about 45-55° C at about 36 mM salt concentration. Specific hybridization as used herein means the use of a final high stringency wash in about 0.2X SSPE (salt concentration of about 36 mM) at a temperature appropriate for the particular primer. 1X SSPE contains 10 mM NaH_2PO_4, 180 mM NaCl, and 1 mM EDTA, at pH 7.4.

The ITS DNA sequences of the present invention can be cloned from fermentation-related microorganisms by methods known in the art. In general, the methods for the isolation of DNA from microorganism isolates are known. See, Raeder & Broda (1985) *Letters in Applied Microbiology* 2:17-20; Lee et al. (1990) *Fungal Genetics Newsletter* 35:23-24; and Lee and Taylor (1990) In: *PCR Protocols: A Guide to Methods and Applications*, Innes et al. (Eds.); pages 282-287; the contents of all of which are hereby incorporated by reference.

Alternatively, the ITS regions of interest can be identified by PCR amplification. Primers to amplify the entire ITS region can be synthesized according to White et al. (1990; In PCR Protocols; Eds.: Innes et al., pages 315-322, the contents of which are hereby incorporated by reference).

The ITS sequences were determined and the sequences were compared to locate divergences which might be useful to test in PCR to distinguish the different fermentation-related microorganisms. The sequences of the ITS regions which were determined are shown as Sequence ID NOS: 1 to 12. The DNA sequences for the ITS1 and ITS2 regions are shown as Sequence ID NOS: 13 to 36. From the identification of divergences, numerous primers were synthesized and tested in PCR-amplification. Purified microorganism

DNA and DNA isolated from fermentation cultures and colonies were used as templates for PCR-amplification. Thus, pairs of diagnostic primers were identified, *i.e.*, those which identified one particular fermentation-related microorganism species. Preferred primer combinations are able to distinguish between the different microorganisms in, for example, fermentation cultures. Primer sequences are set forth in Sequence ID NOS: 65 to 98, with flanking sequences depicted in Sequence ID NOS: 37 to 64. Thus, while oligonucleotide primers selected from among Sequence ID NOS: 65 to 98 are preferred, primers may also be used which contain at least 10 contiguous nucleotide bases from one of SEQ ID NOS: 65 to 98. Additionally, primers may be used which contain at least 10 contiguous nucleotide bases from one of SEQ ID NOS: 65 to 98 contiguous with 1 to 15 nucleotide bases in the 5' and/or 3' direction of corresponding SEQ ID NOS: 37 to 64, and primers of 10 nucleotide bases or longer which contain at least 5 contiguous nucleotide bases from one of SEQ ID NOS: 65 to 98 contiguous with from 1 to 15 nucleotide bases in the 5' and/or 3' direction of corresponding SEQ ID NOS: 37 to 64.

The present invention provides numerous diagnostic primer combinations. The primers of the invention are designed based on sequence differences among the microorganism ITS regions. A minimum of one base pair difference between sequences can permit design of a discriminatory primer. In general, primers should have a theoretical melting temperature between about 55 °C to about 65 °C to achieve good sensitivity, and should be void of significant secondary structure and 3' overlaps between primer combinations. Primers are generally at least about 10 nucleotide bases, more preferably at least about 15 to about 20 nucleotide bases.

The oligonucleotide primers of the present invention are particularly useful in detecting microorganisms involved in fermentations, in particular, microorganisms selected from among *Saccharomyces cerevisiae*, *Saccharomyces bayanus*, *Saccharomycodes ludwigii*, *Dekkera bruxellensis*,

Dekkera intermedia, *Botrytis cinerea*, *Penicillium crustosum*, *Penicillium expansum*, *Hanseniaspora guilliermondii*, *Debaryomyces carsonii*, *Pichia anomala*, *Pichia kluyveri*, and *Candida krusei*. However, the primers of the present invention can also be used to detect the presence of the foregoing microorganisms from any source.

The present invention also relates to the preparation of "kits" containing elements for detecting fermentation-related microorganisms. Such a kit may comprise a carrier to receive therein one or more containers, such as tubes or vials. Unlabeled or detectably labeled oligonucleotide primers may be contained in one or more of the containers. The oligonucleotide primers may be present in lyophilized form, or in an appropriate buffer. One or more enzymes or reagents for use in PCR reactions may be contained in one or more of the containers. The enzymes or reagents may be present alone or in admixture, and in lyophilized form or in appropriate buffers. The kit may also contain any other component necessary for carrying out the present invention, such as buffers, extraction agents, enzymes, pipettes, plates, nucleic acids, nucleoside triphosphates, filter paper, gel materials, transfer materials, and autoradiography supplies.

The examples below illustrate typical experimental protocols which can be used in the isolation of ITS sequences, the selection of suitable primer sequences, the testing of primers for selective and diagnostic efficacy, and the use of such primers to detect the presence of a fermentation-related microorganism. Such examples are provided by way of illustration and not by way of limitation.

Examples

Example 1

Culture of Yeast and Fungal Isolates

Viable isolates of *Botrytis cinerea*, *Penicillium crustosum*, and *Penicillium expansum* were obtained from the American Type Culture Collection (ATCC). Fungi were grown in 40 ml of Malt Yeast Extract Broth in 250 ml flasks inoculated with mycelial fragments from two-week-old cultures grown on Malt Yeast Extract Agar (MYEA). Liquid cultures were incubated at room temperature for 14 days without shaking. Malt Yeast Extract Agar plates were inoculated with mycelia and grown for 2 weeks. Viable isolates of *Saccharomyces cerevisiae*, *Saccharomyces bayanus*, *Saccharomycodes ludwigii*, *Dekkera bruxellensis*, *Dekkera intermedia*, *Dekkera anomala*, *Hanseniaspora guilliermondii*, *Debaryomyces carsonii*, *Pichia anomala*, *Pichia kluyveri*, and *Candida krusei* were obtained from the ATCC or from the Gallo Sonoma Winery (see Table 1). Yeasts were grown on any of several media of choice.

Example 2

Amplification and Sequencing of the Internal Transcribed Spacer (ITS) Regions

The internal transcribed spacer region was amplified from the different isolates directly from the fungal mycelium or the yeast colony using ITS5 (5'-GGAAGTAAAAGTCGTAACAAGG-3'; SEQ ID NO: 99) and ITS4 (5'-TCCTCCGCTTATTGATATGC-3'; SEQ ID NO: 100). A sterile pipette tip was used to scrape a small amount of mycelia or colony off of the plate and deposited into a 250-µl microcentrifuge tube containing 5 µl each of GeneAmp® 10X PCR Buffer II and $MgCl_2$ solution (PE Applied Biosystems, Foster City, CA; part no. N808-0161), 0.2 mM each of dATP, dCTP, dGTP, and dTTP

(GeneAmp® dNTPs; PE Applied Biosystems, Foster City, CA; part no. N808-0007), approximately 25 pmole/μl each of ITS5 and ITS4, and 2.5 Units AmpliTaq® DNA polymerase (PE Applied Biosystems; part no. N808-0161). Reactions were run for 35 cycles of 30 s at 94 °C, 40 s at 58 °C, and 2 min at 72 °C, followed by a final elongation step at 72 °C for 10 min, on a Perkin Elmer GeneAmp® PCR System 9600 (PE Applied Biosystems). PCR products were purified using QIAquick® PCR Purification Kits (Qiagen Inc., Santa Clarita, CA) to remove any excess primers, nucleotides, and polymerases. Five microliters of the purified PCR products were run on a 1.2% agarose gel with 5 μl of pGEM®-3Zf(+) double-stranded DNA Control Template (0.2 g/L, PE Applied Biosystems) to approximate concentrations. All products were sequenced using the primers ITS5 and ITS4 (see sequences above; White et al., 1990; In: *PCR Protocols*; Eds.: Innes et al. pp. 315-322). Sequencing was performed on an PE Applied Biosystems 377 Automated DNA Sequencer® using ABI PRISM™ Dye Terminator Cycle Sequencing Ready Reaction Kits® (PE Applied Biosystems; part no. 402079). Cycle sequencing products were run over Centri-Sep® spin columns (Princeton Separations, Inc., Adelphia, NJ) to remove excess primers, dye-labeled terminators, nucleotides, and polymerases before being run on the automated sequencer.

Example 3

Selection of Species-Specific Primers

The ITS sequences of the *Saccharomyces cerevisiae*, *Saccharomyces bayanus*, *Saccharomycodes ludwigii*, *Dekkera bruxellensis*, *Dekkera intermedia*, *Dekkera anomala*, *Botrytis cinerea*, *Penicillium crustosum*, *Penicillium expansum*, *Hanseniaspora guilliermondii*, *Debaryomyces carsonii*, *Pichia anomala*, *Pichia kluyveri*, and *Candida krusei* isolates were aligned and

primers were designed using Oligo 5.0 (National Biosciences, Inc., Plymouth, MN) in regions of maximum sequence difference between the target species.

Example 4

Primer Synthesis

Primers were synthesized on an Applied Biosystems 394 DNA/RNA Synthesizer® using phosphoramidite chemistry.

Example 5

Verification of Primer Specificity to Target Species

Different annealing temperatures were tested to determine the optimal temperature for PCR for individual primers. In cases with multiple species-specific primers, different primer combinations were used to determine the best primer combination and annealing temperature to amplify a single species-specific DNA fragment. Species-specific amplification products were produced from primers designed from the ITS region between the 18S and 28S ribosomal DNA subunits of each fungal strain of interest.

Example 6

Utilization of ITS sequences as diagnostic probes to hybridize with target DNA

1. Put chosen concentration of DNA sample in 100 ul of TE, pH 7.0.
2. Add 0.1 volume [10 μl] of 3.0 M NaOH, vortex to mix and incubate at

65° C for 20 min to destroy the RNA and denature the DNA.

3. Spin down condensation. Allow samples to cool to room temp. Neutralize by adding 1.0 volume [110 μl] of 2M ammonium acetate, pH 7.0,

vortex to mix. Spin down to remove solution off of cap. Refrigerate until slot blot apparatus is ready.

4. Apply to slot-blot apparatus according to manufacturers protocol; about 220 μl to slot blot.

5. Label ITS sequence probe according to kit manufacturer's recommendation.

6. Prehybridize blots in 1.0% BSA; 1mM EDTA, 0.5 M $NaHPO_4$, pH 7.2, 7.0% sodium dodecyl sulfate for a minimum of 2 hr prior to adding the probe, and then hybridized for 16 hr at 45° C. Initial washes consist of two 30-min washes in 1X SSPE/0.1% SDS at 50° C. Transfer blots to a plastic tray and wash in 1X SSPE for 1 hr, at 50° C with shaking. The final wash should consist of 15 min at 50° C in 0.2X SSPE.

While the invention has been described in detail and with reference to specific embodiments thereof, it will be apparent to one skilled in the art that various changes and modifications can be made therein without departing from the spirit and scope thereof.

Table 1

Sources of test isolates

Species name	ID number	Source
Saccharomyces cerevisiae	GS061	Gallo of Sonoma[1]
Saccharomyces cerevisiae	GS117	Gallo of Sonoma
Saccharomyces cerevisiae	4127	ATCC[2]
Saccharomyces bayanus	13056	ATCC
Saccharomycodes ludwigii	34085	ATCC
Saccharomycodes ludwigii	44299	ATCC
Dekkera bruxellensis	Y153	Gallo of Sonoma
Dekkera bruxellensis	Y207	Gallo of Sonoma
Dekkera bruxellensis	10560	ATCC
Dekkera intermedia	34448	ATCC
Dekkera anomala	10559	ATCC
Botrytis cinerea	20599	ATCC
Penicillium crustosum	58616	ATCC
Penicillium expansum	28885	ATCC
Hanseniaspora guilliermondii	GS014	Gallo of Sonoma
Hanseniaspora guilliermondii	GS057	Gallo of Sonoma
Hanseniaspora guilliermondii	66166	ATCC
Debaryomyces carsonii	Y443	Gallo of Sonoma
Debaryomyces carsonii	Y448	Gallo of Sonoma
Debaryomyces carsonii	24214	ATCC
Pichia anomala	GS085	Gallo of Sonoma
Pichia anomala	34080	ATCC
Pichia kluyveri	GS070	Gallo of Sonoma
Pichia kluyveri	64303	ATCC
Candida krusei	GS076	Gallo of Sonoma
Candida krusei	GS096	Gallo of Sonoma
Candida krusei	GS108	Gallo of Sonoma

[1]Gallo of Sonoma Winery, Healdsburg, CA, USA

[2]American Type Culture Collection, Rockville, MD, USA

Table 2

Target Organism	Primer Name	Primer Sequence
Saccharomyces sp.	SXU99	5'-CTTTTACTGGGCAAGAAGAC-3' (SEQ ID NO: 65)
Saccharomyces sp.	SXL658	5'-AAGCACGCAGAGAAACC-3' (SEQ ID NO: 66)
Saccharomyces sp.	SXU102	5'-TTACTGGGCAAGAAGACAAG-3' (SEQ ID NO: 67)
Saccharomyces sp.	SXL661	5'-CTCAAGCACGCAGAGAA-3' (SEQ ID NO: 68)
Saccharomycodes ludwigii	SLU85	5'-GACTTTTCTTGGGGAGAG-3' (SEQ ID NO: 69)
Saccharomycodes ludwigii	SLL635	5'-TCACTAGTTGGGATAAACCT-3' (SEQ ID NO: 70)
Saccharomycodes ludwigii	SLU88	5'-TTTTCTTGGGGAGAGG-3' (SEQ ID NO: 71)
Saccharomycodes ludwigii	SLL636	5'-ATCACTAGTTGGGATAAACC-3' (SEQ ID NO: 72)
Saccharomycodes ludwigii	SLU136	5'-CGGCTAGTAGTTGATGATT-3' (SEQ ID NO: 73)
Saccharomycodes ludwigii	SLL634	5'-CACTAGTTGGGATAAACCTA-3' (SEQ ID NO: 74)
Dekkera sp.	BRU53A	5'-ATTACAGGATGCTGGGC-3' (SEQ ID NO: 75)
Dekkera sp.	BRU53B	5'-ATTACAGGATGCTGGG-3' (SEQ ID NO: 76)
Dekkera sp.	BRU77	5'-CGTGCAGACACGTGGAT-3' (SEQ ID NO: 77)
Dekkera sp.	BRL339	5'-CTTTGAAGAAAACACCCTC-3' (SEQ ID NO: 78)
Dekkera sp.	BRL367	5'-AATCATTATCCCCTCACTC-3' (SEQ ID NO: 79)

Target Organism	Primer Name	Primer Sequence
Dekkera sp.	BRL390	5'-TAATGAACGGCCGAAAC-3' (SEQ ID NO: 80)
Botrytis cinerea	BCU136	5'-TTGTATGCTCGCCAGAG-3' (SEQ ID NO: 81)
Botrytis cinerea	BCL393	5'-GCCTGCCATTACTGACA-3' (SEQ ID NO: 82)
Botrytis cinerea	BCU142	5'-GCTCGCCAGAGAATACC-3' (SEQ ID NO: 83)
Penicillium sp.	PXU87	5'-ACCCGTCTTTATTTTACCTT-3' (SEQ ID NO: 84)
Penicillium sp.	PXL495	5'-CTACAGAGCGGGTGACA-3' (SEQ ID NO: 85)
Penicillium sp.	PXU86	5'-CACCCGTGTTTATTTTACCT-3' (SEQ ID NO: 86)
Penicillium sp.	PXL482	5'-CAAAGCCCCATACGC-3' (SEQ ID NO: 87)
Penicillium sp.	PXL491	5'-AGCGGGTGACAAAGC-3' (SEQ ID NO: 88)
Hanseniaspora guilliermondii	HGU193	5'-CAACGTTTACACACATTGG-3' (SEQ ID NO: 89)
Hanseniaspora guilliermondii	HGU231	5'-AATTCTTTCTGCTTTGAATCG-3' (SEQ ID NO: 90)
Hanseniaspora guilliermondii	HGL601	5'-CAGCGTCTCCAAAGAAGCTAA-3' (SEQ ID NO: 91)
Debaryomyces carsonii	DXL447	5'-GCAAACGCCTAGTTCGACTAA-3' (SEQ ID NO: 92)
Debaryomyces carsonii	DXL526	5'-ATTCAACGAGTTGGATAAACC-3' (SEQ ID NO: 93)
Pichia anomala	PAU133	5'-GGCTTACTGCCCAAAGGTC-3' (SEQ ID NO: 94)
Pichia anomala	PAL451	5'-TGCTTATTAGTACACTCTTGC-3' (SEQ ID NO: 95)

Target Organism	Primer Name	Primer Sequence
Pichia kluyveri	PKL356	5'-GTTTAGTTCACTTCGTCCACG-3' (SEQ ID NO: 96)
Candida krusei	CKU104	5'-CCTAAAATGTGGAATATAGCA-3' (SEQ ID NO: 97)
Candida krusei	CKL354	5'-ACGCTCTTTACACGTCGTC-3' (SEQ ID NO: 98)

Table 3

ITS-derived Diagnostic PCR Primers

Target Organism	5' Primer	3' Primer	Approximate Size of PCR Product (bp)
Saccharomyces sp.	SXU99	ITS4	750
Saccharomyces sp.	SXU102	ITS4	750
Saccharomyces sp.	ITS5	SXL658	695
Saccharomyces sp.	ITS5	SXL661	695
Saccharomyces sp.	SXU99	SXL658	560
Saccharomyces sp.	SXU99	SXL661	560
Saccharomyces sp.	SXU102	SXL658	560
Saccharomyces sp.	SXU102	SXL661	560
Saccharomycodes ludwigii	SLU85	ITS4	660
Saccharomycodes ludwigii	SLU88	ITS4	660
Saccharomycodes ludwigii	SLU136	ITS4	610
Saccharomycodes ludwigii	ITS5	SLL635	670
Saccharomycodes ludwigii	ITS5	SLL636	670
Saccharomycodes ludwigii	ITS5	SLL634	670
Saccharomycodes ludwigii	SLU85	SLL635	565
Saccharomycodes ludwigii	SLU85	SLL636	565
Saccharomycodes ludwigii	SLU85	SLL634	565
Saccharomycodes ludwigii	SLU88	SLL635	565
Saccharomycodes ludwigii	SLU88	SLL636	565
Saccharomycodes ludwigii	SLU88	SLL634	565
Saccharomycodes ludwigii	SLU136	SLL635	500
Saccharomycodes ludwigii	SLU136	SLL636	500
Saccharomycodes ludwigii	SLU136	SLL634	500
Dekkera sp.	BRU53A	ITS4	450
Dekkera sp.	BRU53B	ITS4	450
Dekkera sp.	BRU77	ITS4	450
Dekkera sp.	ITS5	BRL339	375
Dekkera bruxellensis	ITS5	BRL367	400
Dekkera sp.	ITS5	BRL390	425
Dekkera sp.	BRU53A	BRL339	290
Dekkera bruxellensis	BRU53A	BRL367	320
Dekkera sp.	BRU53A	BRL390	340

Dekkera sp.	BRU53B	BRL339	290
Dekkera bruxellensis	BRU53B	BRL367	320
Dekkera sp.	BRU53B	BRL390	340
Dekkera sp.	BRU77	BRL339	260
Dekkera bruxellensis	BRU77	BRL367	290
Dekkera sp.	BRU77	BRL390	310
Botrytis cinerea	BCU136	ITS4	350
Botrytis cinerea	BCU142	ITS4	345
Botrytis cinerea	ITS5	BCL393	420
Botrytis cinerea	BCU136	BCL393	270
Botrytis cinerea	BCU142	BCL393	265
Penicillium sp.	PXU87	ITS4	410
Penicillium sp.	PXU86	ITS4	410
Penicillium sp.	ITS5	PXL495	530
Penicillium sp.	ITS5	PXL482	520
Penicillium sp.	ITS5	PXL491	530
Penicillium sp.	PXU87	PXL495	405
Penicillium sp.	PXU87	PXL482	395
Penicillium sp.	PXU87	PXL491	405
Hanseniaspora guilliermondii	HGU193	ITS4	530
Hanseniaspora guilliermondii	HGU231	ITS4	490
Hanseniaspora guilliermondii	ITS5	HGL601	630
Hanseniaspora guilliermondii	HGU193	HGL601	420
Hanseniaspora guilliermondii	HGU231	HGL601	380
Debaryomyces carsonii	ITS5	DXL447	480
Debaryomyces carsonii	ITS5	DXL526	560
Pichia anomala	PAU133	ITS4	505
Pichia anomala	ITS5	PAL451	480
Pichia anomala	PAU133	PAL451	320
Pichia kluyveri	ITS5	PKL356	390
Candida krusei	CKU104	ITS4	440
Candida krusei	ITS5	CKL354	385

We claim:

1. An isolated double stranded nucleic acid selected from the group consisting of SEQ ID NOS: 13 to 36.

2. An isolated nucleic acid which specifically hybridizes with the nucleic acid of claim 1.

3. An oligonucleotide sequence for identification of a fermentation-related microorganism, wherein said sequence is selected from the group consisting of SEQ ID NOS: 37 to 64.

4. An oligonucleotide primer which is a fragment of the sequences according to claim 3, and which specifically hybridizes to the ITS1 or ITS2 of *Saccharomyces cerevisiae*, *Saccharomyces bayanus*, *Saccharomycodes ludwigii*, *Dekkera bruxellensis*, *Dekkera intermedia*, *Botrytis cinerea*, *Penicillium crustosum*, *Penicillium expansum*, *Hanseniaspora guilliermondii*, *Debaryomyces carsonii*, *Pichia anomala*, *Pichia kluyveri* or *Candida krusei*.

5. An oligonucleotide primer for identification of a fermentation-related microorganism, wherein said primer is selected from the group consisting of primers which contain at least 10 contiguous nucleotide bases from one of SEQ ID NOS: 65 to 98, primers which contain at least 10 contiguous nucleotide bases from one of SEQ ID NOS: 65 to 98 contiguous with 1 to 15 nucleotide bases in the 5' and/or 3' direction of corresponding SEQ ID NOS: 37 to 64, and primers of 10 nucleotide bases or longer which contain at least 5 contiguous nucleotide bases from one of SEQ ID NOS: 65 to 98 contiguous with from 1 to 15 nucleotide bases in the 5' and/or 3' direction of corresponding SEQ ID NOS: 37 to 64.

6. A pair of oligonucleotide primers for use in the amplification-based detection of an internal transcribed spacer sequence of a fermentation-related microorganism, wherein the primers are selected from the group consisting of primers which contain at least 10 contiguous nucleotide bases from one of SEQ ID NOS: 65 to 98, primers which contain at least 10 contiguous nucleotide bases from one of SEQ ID NOS: 65 to 98 contiguous with 1 to 15 nucleotide bases in the 5' and/or 3' direction of corresponding SEQ ID NOS: 37 to 64, and primers of 10 bases or longer which contain at least 5 contiguous nucleotide bases from one of SEQ ID NOS: 65 to 98 contiguous with from 1 to 15 nucleotide bases in the 5' and/or 3' direction of corresponding SEQ ID NOS: 37 to 64.

7. The pair of oligonucleotide primers according to claim 6 which comprises SEQ ID NO: 65 and SEQ ID NO: 66.

8. The pair of oligonucleotide primers according to claim 6 which comprises SEQ ID NO: 67 and SEQ ID NO: 68.

9. The pair of oligonucleotide primers according to claim 6 which comprises SEQ ID NO: 69 and SEQ ID NO: 70.

10. The pair of oligonucleotide primers according to claim 6 which comprises SEQ ID NO: 71 and SEQ ID NO: 72.

11. The pair of oligonucleotide primers according to claim 6 which comprises SEQ ID NO: 73 and SEQ ID NO: 74.

12. The pair of oligonucleotide primers according to claim 6 which comprises SEQ ID NO: 75 and SEQ ID NO: 78.

13. The pair of oligonucleotide primers according to claim 6 which comprises SEQ ID NO: 76 and SEQ ID NO: 79.

14. The pair of oligonucleotide primers according to claim 6 which comprises SEQ ID NO: 77 and SEQ ID NO: 80.

15. The pair of oligonucleotide primers according to claim 6 which comprises SEQ ID NO: 81 and SEQ ID NO: 82.

16. The pair of oligonucleotide primers according to claim 6 which comprises SEQ ID NO: 85 and SEQ ID NO: 83.

17. The pair of oligonucleotide primers according to claim 6 which comprises SEQ ID NO: 84 and SEQ ID NO: 85.

18. The pair oligonucleotide primers according to claim 6 which comprises SEQ ID NO: 86 and SEQ ID NO: 87.

19. The pair of oligonucleotide primers according to claim 6 which comprises SEQ ID NO: 86 and SEQ ID NO: 88.

20. The pair of oligonucleotide primers according to claim 6 which comprises SEQ ID NO: 89 and SEQ ID NO: 91.

21. The pair of oligonucleotide primers according to claim 6 which comprises SEQ ID NO: 90 and SEQ ID NO: 91.

22. The pair oligonucleotide primers according to claim 6 which comprises SEQ ID NO: 94 and SEQ ID NO: 95.

23. The pair of oligonucleotide primers according to claim 6 which comprises SEQ ID NO: 97 and SEQ ID NO: 98.

24. A method for detection of a fermentation-related microorganism comprising:

(a) obtaining DNA from a fungal culture or colony isolated from a fermentation, or from an organism present in a fermentation beverage;

(b) amplifying a part of the internal transcribed spacer sequence of said fermentation-related microorganism using said DNA as a template in a polymerase chain reaction with a pair of oligonucleotide primers according to claim 6; and

(c) visualizing said amplified part of the internal transcribed spacer sequence to determine whether said fermentation-related microorganism is present.

25. The method according to claim 24, wherein said fermentation-related microorganism is selected from the group consisting of *Saccharomyces cerevisiae*, *Saccharomyces bayanus*, *Saccharomycodes ludwigii*, *Dekkera bruxellensis*, *Dekkera intermedia*, *Botrytis cinerea*, *Penicillium crustosum*, *Penicillium expansum*, *Hanseniaspora guilliermondii*, *Debaryomyces carsonii*, *Pichia anomala*, *Pichia kluyveri*, and *Candida krusei*.

26. The method according to claim 24, wherein said fermentation culture or fermentation beverage is a wine fermentation culture or wine fermentation beverage.

27. The method according to claim 24, wherein the pair of oligonucleotide primers comprises SEQ ID NO: 65 and SEQ ID NO: 66.

28. The method according to claim 24, wherein the pair of oligonucleotide primers comprises SEQ ID NO: 67 and SEQ ID NO: 68.

29. The method according to claim 24, wherein the pair of oligonucleotide primers comprises SEQ ID NO: 69 and SEQ ID NO: 70.

30. The method according to claim 24, wherein the pair of oligonucleotide primers comprises SEQ ID NO: 71 and SEQ ID NO: 72.

31. The method according to claim 24, wherein the pair of oligonucleotide primers comprises SEQ ID NO: 73 and SEQ ID NO: 74.

32. The method according to claim 24, wherein the pair of oligonucleotide primers comprises SEQ ID NO: 75 and SEQ ID NO: 78.

33. The method according to claim 24, wherein the pair of oligonucleotide primers comprises SEQ ID NO: 76 and SEQ ID NO: 79.

34. The method according to claim 24, wherein the pair of oligonucleotide primers comprises SEQ ID NO: 77 and SEQ ID NO: 80.

35. The method according to claim 24, wherein the pair of oligonucleotide primers comprises SEQ ID NO: 81 and SEQ ID NO: 82.

36. The method according to claim 24, wherein the pair of oligonucleotide primers comprises SEQ ID NO: 82 and SEQ ID NO: 83.

37. The method according to claim 24, wherein the pair of oligonucleotide primers comprises SEQ ID NO: 84 and SEQ ID NO: 85.

38. The method according to claim 24, wherein the pair of oligonucleotide primers comprises SEQ ID NO: 86 and SEQ ID NO: 87.

39. The method according to claim 24, wherein the pair of oligonucleotide primers comprises SEQ ID NO: 86 and SEQ ID NO: 88.

40. The method according to claim 24, wherein the pair of oligonucleotide primers comprises SEQ ID NO: 89 and SEQ ID NO: 91.

41. The method according to claim 24, wherein the pair of oligonucleotide primers comprises SEQ ID NO: 90 and SEQ ID NO: 91.

42. The method according to claim 24, wherein the pair of oligonucleotide primers comprises SEQ ID NO: 94 and SEQ ID NO: 95.

43. The method according to claim 24, wherein the pair of oligonucleotide primers comprises SEQ ID NO: 97 and SEQ ID NO: 98.

44. A kit comprising a carrier to receive therein one or more containers, at least one of said containers comprising an oligonucleotide primer according to claim 5.

45. A kit comprising a carrier to receive therein one or more containers, at least one of said containers comprising a pair of oligonucleotide primers according to claim 6.

Sequence Listing

SEQ ID NO: 1 DNA sequence for the internal transcribed spacer of *Saccharomyces cerevisiae* and *Saccharomyces bayanus*.

5' AAGAAATTTAATAATTTTGAAAATGGATTTTTTTGTTTT
GGCAAGAGCATGAGAGCTTTTACTGGGCAAGAAGACAA
GAGATGGAGAGTCCACCCGGGCCTGCGCTTAAGTGCGC
GGTCTTGCTAGGCTTGTAAGTTTCTTTCTTGCTATTCCA
AACGGTGAGAGATTTCTGTGCTTTTGTTATAGGACAATT
AAAACCGTTTCAATACAACACACTGTGGAGTTTTCATAT
CTTTGCAACTTTTTCTTTGGGCATTCGAGCAATCGGGGC
CCAGAGGTAACAAACACAAACAATTTTATCTATTCATTA
AATTTTTGTCAAAAACAAGAATTTTCGTAACTGGAAATT
TTAAAATATTAAAAACTTTCAACAACGGATCTCTTGGTT
CTCGCATCGATGAAGAACGCAGCGAAATGCGATACGTA
ATGTGAATTGCAGAATTCCGTGAATCATCGAATCTTTGA
ACGCACATTGCGCCCCTTGGTATTCCAGGGGGCATGCCT
GTTTGAGCGTCATTTCCTTCTCAAACATTCTGTTTGGTA
GTGAGTGATACTCTTTGGAGTTAACTTGAAATTGCTGGC
CTTTTCATTGGATGTTTTTTTTTCCAAAGAGAGGTTTCTC
TGCGTGCTTGAGGTATAATGCAAGTACGGTCGTTTTAGG
TTTTACCAACTGCGGCTAATCTTTTTTTATACTGAGCGT
ATTGGAACGTTATCGATAAGAAGAGAGCGTCTAGGCGA
ACAATGTTCTTAAAGT 3'

SEQ ID NO: 2 DNA sequence for the internal transcribed spacer of *Saccharomycodes ludwigii*.

5' AAGAAAAAAACTGTTTATAAACAGACGGTAGACTTTTC
TTGGGGAGAGGTTGTTGATTGCTTTGGCCTGCGCTTAAC
TGCGCGGCTAGTAGTTGATGATTTTGTTATTATCCGAGA
CGAAGGAAACGTCTGATTTTAAAAACATTATACACTTTG
GAGTACTTTTTTTAATGTATTTCTTCCTTGGACGAGCAA
TTGTTCAAGGGTCAATAAACACAAACAATTTTTTTTTTA
TTTAAATTTAAAATAATTCAAAATATATCATTTCTTTTA
TTAGGAATATAAAAATTTTAAAACTTTCAACAACGGATC
TCTTGGTTCTCGCATCGATGAAGAACGTAGCGAATTGCG
ATAAGTAATGTGAATTGCAGATTTTCGTGAATCATTGAA
TTTTTGAACGCACATTGCGCCCTTTGGTATTCCAAAGGG
CATGCCTGTTTGAGCGTCATTTCCTTCTCAAAAGAGTTT
TTTTATTCTTTTGGTTGTGAGTGATACTCTTTCCTTTTAC

AGGGAAGGGGTTAACTTGAAATTGTTGCCTAGCAAAGA
AGAATTTTGATTGAAATTTCTTGTTTATTACTATTAGGT
TTATCCCAACTAGTGATTATTGAGAGTTTTTATTACAGA
GTCTTTTCACTTGCTATAATACTATTCTATAAGT 3'

SEQ ID NO: 3 DNA sequence for the internal transcribed spacer of *Dekkera bruxellensis*.

5' CAGGATGCTGGGCGCAAGCCCGTGCAGACACGTGGATA
AGCAAGGATAAAAATACATTAAATTTATTTAGTTTAGTC
AAGAAAGAATTTTAAAACTTTCAACAATGGATCTCTTGG
TTCTCGCGTCGATGAAGAGCGCAGCGGAATTGCGATAC
TTAATGTGAATTGCAGATTTTCGTGAATCATCGAGTTCT
TGAACGCACATTGCGCCCTCTGGTATTCCGGAGGGCAT
GCCTGTTTGAGCGTCATTTCCTTCTCACTATTTAGTGGT
TATGAGATTACACGAGGGTGTTTTCTTCAAAGGAAAGA
GGGGAGAGTGAGGGGATAATGATTTAAGGTTTCGGCCG
TTCATTATTTTTTCTTCTCCCCCAGTTATCAAGT 3'

SEQ ID NO: 4 DNA sequence for the internal transcribed spacer of *Dekkera intermedia*.

5' CAGGATGCTGGGCGCAAGCCCGTGCAGACACGTGGATA
AGTAAGGATAAAAATACATTAAATTTATTTAGTTTTAGT
CAAGAAAGAATTTTAAAACTTTCAACAATGGATCTCTTG
GTTCTCGCGTCGATGAAGAGCGCAGCGAATTGCGATAC
TTAATGTGAATTGCAGATTTTCGTGAATCATCGAGTTCT
TGAACGCACATTGCGCCCTCTGGTATTCCGGAGGGCAT
GCCTGTTTGAGCGTCATTTCCTTCTCACTATTTAGTGGT
TATGAGATTACACGAGGGTGTTTTCTTCAAAGGAAAGA
GGGGAGAGTGAGGGGATAATGATTTAAGGTTTCGGCCG
TTCATTATTTTTTCTTCTCCCCCAGTTATCAAGT 3'

SEQ ID NO: 5 DNA sequence for the internal transcribed spacer of *Botrytis cinerea*.

5' CAGAGTTCATGCCCGAAAGGGTAGACCTCCCACCCTTG
TGTATTATTACTTTGTTGCTTTGGCGAGCTGCTTTCGGG
CCTTGTATGCTCGCCAGAGAATACCAAAACTCTTTTTAT
TAATGTCGTCTGAGTACTATATAATAGTTAAAACTTTCA

ACAACGGATCTCTTGGTTCTGGCATCGATGAAGAACGC
AGCGAAATGCGATAAGTAATGTGAATTGCAGAATTCAG
TGAATCATCGAATCTTTGAACGCACATTGCGCCCCTTGG
TATTCCGGGGGGGCATGCCTGTTCGAGCGTCATTTCAACC
CTCAAGCTTAGCTTGGTATTGAGTCTATGTCAGTAATGG
CAGGCTCTAAAATCAGTGGCGGCGCCGCTGGGTCCTGA
ACGTAGTAATATCTCTCGTTACAGGTTCTCGGTGTGCTT
CTGCCAAAACCCAAATTTTTCTATGG 3'

SEQ ID NO: 6 DNA sequence for the internal transcribed spacer of *Penicillium crustosum*.

5' CCGAGTGAGGGCCCTCTGGGTCCAACCTCCCACCCGTG
TTTATTTTACCTTGTTGCTTCGGCGGGCCCGCCTTAACT
GGCCGCCGGGGGGGCTTACGCCCCCGGGCCCGCGCCCGC
CGAAGACACCCTCGAACTCTGTCTGAAGATTGAAGTCT
GAGTGAAAATATAAATTATTTAAAACTTTCAACAACGG
ATCTCTTGGTTCCGGCATCGATGAAGAACGCAGCGAAA
TGCGATACGTAATGTGAATTGCAAATTCAGTGAATCATC
GAGTCTTTGAACGCACATTGCGCCCCCTGGTATTCCGGG
GGGCATGCCTGTCCGAGCGTCATTGCTGCCCTCAAGCCC
GGCTTGTGTGTTGGGCCCCGTCCCCCGATCTCCGGGGGA
CGGGCCCGAAAGGCAGCGGCGGCACCGCGTCCGGTCCT
CGAGCGTATGGGGCTTTGTCACCCGCTCTGTAGGCCCGG
CCGGCGCTTGCCGATCAACCCAAATTTTTATCCAGG 3'

SEQ ID NO: 7 DNA sequence for the internal transcribed spacer of *Penicillium expansum*.

5' CCGAGTGAGGGCCCTTTGGGTCCAACCTCCCACCCGTGT
TTATTTACCTCGTTGCTTCGGCGGGCCCGCCTTAACTGG
CCGCCGGGGGGCTCACGCCCCCGGGCCCGCGCCCGCCG
AAGACACCCCCGAACTCTGCCTGAAGATTGTCGTCTGA
GTGAAAATATAAATTATTTAAAACTTTCAACAACGGATC
TCTTGGTTCCGGCATCGATGAAGAACGCAGCGAAATGC
GATACGTAATGTGAATTGCAAATTCAGTGAATCATCGA
GTCTTTGAACGCACATTGCGCCCCCTGGTATTCCGGGGG
GCATGCCTGTCCGAGCGTCATTGCTGCCCTCAAGCCCGG
CTTGTGTGTTGGGCCCCGTCCTCCGATTCCGGGGGACGG
GCCCGAAAGGCAGCGGCGGCACCGCGTCCGGTCCTCGA
GCGTATGGGGCTTTGTCACCCGCTCTGTAGGCCCGGCCG
GCGCTTGCCGATCAACCCAAATTTTTATCCAGGTTGACC

TCGGATCAGGTAGGGATACCCGCTGAACTTAAGCATAT
CAATAAGCGGAGGAAA 3'

SEQ ID NO: 8 DNA sequence for the internal transcribed spacer of *Hanseniaspora guilliermondii*.

5' GATTGAATTATCATTGTTGCTCGAGTTCTAGTTTTAGAT
CTTTTACAATAATGTGTATCTTTATTGAAGATGTGCGCT
TAATTGCGCTGCTTTTTTAAAGTGTCGCAGTAGAAGTAA
TCTTGCTTGAATCTCAGTCAACGTTTACACACATTGGAG
TTTTTTTACTTTAATTTAATTCTTTCTGCTTTGAATCGAA
AGGTTCAAGGCAAAAAACAAACACAAACAATTTTATTT
TATTATAATTTTTTAAACTAAACCAAAATTCCTAACGGA
AATTTTAAAATAATTTAAAACTTTCAACAACGGATCTCT
TGGTTCTCGCATCGATGAAGAACGTAGCGAATTGCGAT
AAKTAATGTGAATTGCAGATACTCGTGAATCATTGAATT
TTTGAACGCACATTGCGCCCTTGAGCATTCTCAAGGGCA
TGCCTGTTTGAGCGTCATTTCCTTCTCAAAAGATAATTT
TTTATTTTTTGGTTGTGGGCGATACTCAGGGTTAGCTTG
AAATTGAAGATTGTTTCAATCTTTTTTAATTCAACACTT
AGCTTCTTTGGAGACGCTGTTCTCGCTGTGATGTATTTA
TGAATTTATTCGTTTTACTTTACAAGGGAAATGGTAATG
TACCTTAGGCAAAGGGTTGCTTTTAATATTCATCAAGT
3'

SEQ ID NO: 9 DNA sequence for the internal transcribed spacer of *Debaryomyces carsonii*.

5' CAGTATTCTTTTTGCCAGCGCTTAACTGCGCGGCGAAAT
AAACCTTACACACAATGTTTTTTGTTATTACAGGAACTT
TTGCTTTGGCTTGTCTCTAGAAATAGAGTTGGGCCAAAG
GTTTAAACTAACTTCAATTTATTTGAACTATTTTTCTTAT
TGAAATGTCACTTTGTTGATTAAATTCAAAAAATCTTCA
AAACTTTCAACAACGGATCTCTTGGTTCTCGCATCGATG
AAGAACGCAGCGAAATGCGATAAGTAATATGAATTGCA
GATTTTCGTGAATCATCGAATCTTTGAACGCACATTGCG
CCCTTTGGTATTCCAAAGGGCATGCCTGTTTGAGCGTCA
TTTCTCTCTCAAACCTTAGGGTTTGGTATTGAGTGATAC
TCTTAGTCGAACTAGGCGTTTGCTTGAAATGTATCGGCA
TGAGTGGTACTAGATTAGTGCTTCAGATTTTTCAATGTA
TTAGGTTTATCCAACTCGTTGAATAGTCTGATGGCAAGT

GTTTAGTAACTATGGCTCGGCCTAACAACAACAAACAA
GT 3'

SEQ ID NO: 10 DNA sequence for the internal transcribed spacer of *Pichia anomala*.

5' TAGTATTCTATTGCCAGCGCTTAATTGCGCGGCGATAAA
CCTTACACACATTGTCTAGTTTTTTTGAACTTTGCTTTGG
GTGGTGAGCCTGGCTTACTGCCCAAAGGTCTAAACACA
TTTTTTTTAATGTTAAAACCTTTAACCAATAGTCATGAAA
ATTTTTAACAAAAATTAAAATCTTCAAAACTTTCAACAA
CGGATCTCTTGGTTCTCGCAACGATGAAGAACGCAGCG
AAATGCGATACGTATTGTGAATTGCAGATTTTCGTGAAT
CATCGAATCTTTGAACGCACATTGCACCCTCTGGTATTC
CAGAGGGTATGCCTGTTTGAGCGTCATTTCTCTCTCAAA
CCTTCGGGTTTGGTATTGAGTGATACTCTGTCAAGGGTT
AACTTGAAATATTGACTTAGCAAGAGTGTACTAATAAG
CAGTCTTTCTGAAATAATGTATTAGGTTCTTCCAACTCG
TTATATCAGCTAGGCAGGTTTAGAAGTATTTTAGGCTCG
GCTTAACAACAATAAACTAAAAGT 3'

SEQ ID NO: 11 DNA sequence for the internal transcribed spacer of *Pichia kluyveri*.

5' CTGTGATTTATATCTTATACACATGCGTGAGCGCACCAA
ACACCTAAAATTGTAATAATACCAGTCACTAAGTTTTAA
CAAAACAAAACTTTCAACAACGGATCTCTTGGTTCTCGC
ATCGATGAAGAGCGCAGcGAAATGCGATACCTAGTGTG
AATTGCAGCCATCGTGAATCATCGAGTTCTTGAACGCAC
ATTGCGCCCCATGGTATTCCATGGGGCATGCCTGTCTGA
GCGTCGTTTCCTTCTTGCGCAAGCAGAGTTGAGAACAG
GCTATGCCTTTTTCGAAATGGAACGTCGTGGACGAAGT
GAACTAAACTTTTAGCACGCTTTGGCCGCCGAACTTTTA
ACTAAGC 3'

SEQ ID NO: 12 DNA sequence for the internal transcribed spacer of *Candida krusei*.

5' CTGTGATTTACTACTACACTGCGTGAGCGGAACGAAAA
CAACAACACCTAAAATGTGGAATATAGCATATAGTCGA

CAAGAGAAATCTACGAAAAAACAAACAAAACTTTCAAC
AACGGATCTCTTGGTTCTCGCATCGATGAAGAGCGCAG
CGAAATGCGATACCTAGTGTGAATTGCAGCCATCGTGA
ATCATCGAGTTCTTGAACGCACATTGCGCCCCTCGGCAT
TCCGGGGGGCATGCCTGTTTGAGCGTCGTTTCCATCTTG
CGCGTGCGCAGAGTTGGGGGAGCGGAGCGGACGACGTG
TAAAGAGCGTCGGAGCTGCGACTCGCCTGAAAGGGAGC
GAAGCTGGCCGAGCGAACTAGACTTTTTTTCAGGGACG
CTTGGCGGCCGAGAGCGAGTGTTGCGAGACAACAAAAA
GC 3'

SEQ ID NO: 13 DNA sequence for the ITS1 of *Saccharomyces cerevisiae* and *Saccharomyces bayanus*.

5' AAGAAATTTAATAATTTTGAAAATGGATTTTTTTGTTTT
GGCAAGAGCATGAGAGCTTTTACTGGGCAAGAAGACAA
GAGATGGAGAGTCCACCCGGGCCTGCGCTTAAGTGCGC
GGTCTTGCTAGGCTTGTAAGTTTCTTTCTTGCTATTCCA
AACGGTGAGAGATTTCTGTGCTTTTGTTATAGGACAATT
AAAACCGTTTCAATACAACACACTGTGGAGTTTTCATAT
CTTTGCAACTTTTTCTTTGGGCATTCGAGCAATCGGGGC
CCAGAGGTAACAAACACAAACAATTTTATCTATTCATTA
AATTTTTGTCAAAAACAAGAATTTTCGTAACTGGAAATT
TTAAAATATTAA 3'

SEQ ID NO: 14 DNA sequence for the ITS2 of *Saccharomyces cerevisiae* and *Saccharomyces bayanus*.

5' CCTTCTCAAACATTCTGTTTGGTAGTGAGTGATACTCTT
TGGAGTTAACTTGAAATTGCTGGCCTTTTCATTGGATGT
TTTTTTTTCCAAAGAGAGGTTTCTCTGCGTGCTTGAGGT
ATAATGCAAGTACGGTCGTTTTAGGTTTTACCAACTGCG
GCTAATCTTTTTTTTATACTGAGCGTATTGGAACGTTATC
GATAAGAAGAGAGCGTCTAGGCGAACAATGTTCTTAAA
GT 3'

SEQ ID NO: 15 DNA sequence for the ITS1 of *Saccharomycodes ludwigii*.

5' AAGAAAAAAACTGTTTATAAACAGACGGTAGACTTTTC
TTGGGGAGAGGTTGTTGATTGCTTTGGCCTGCGCTTAAC
TGCGCGGCTAGTAGTTGATGATTTTGTTATTATCCGAGA
CGAAGGAAACGTCTGATTTTAAAAACATTATACACTTTG
GAGTACTTTTTTTAATGTATTTCTTCCTTGGACGAGCAA
TTGTTCAAGGGTCAATAAACACAAACAATTTTTTTTTTA
TTTAAATTTAAAATAATTCAAAATATATCATTTCTTTTA
TTAGGAATATAAAAATTTTA 3'

SEQ ID NO: 16 DNA sequence for the ITS2 of *Saccharomycodes ludwigii*.

5' CCTTCTCAAAAGAGTTTTTTTATTCTTTTGGTTGTGAGTG
ATACTCTTTCCTTTTACAGGGAAGGGGTTAACTTGAAAT
TGTTGCCTAGCAAAGAAGAATTTTGATTGAAATTTCTTG
TTTATTACTATTAGGTTTATCCCAACTAGTGATTATTGA
GAGTTTTTATTACAGAGTCTTTTCACTTGCTATAATACT
ATTCTATAAGT 3'

SEQ ID NO: 17 DNA sequence for the ITS1 of *Dekkera bruxellensis*.

5' CAGGATGCTGGGCGCAAGCCCGTGCAGACACGTGGATA
AGCAAGGATAAAAATACATTAAATTTATTTAGTTTAGTC
AAGAAAGAATTTTA 3'

SEQ ID NO: 18 DNA sequence for the ITS2 of *Dekkera bruxellensis*.

5' CCTTCTCACTATTTAGTGGTTATGAGATTACACGAGGGT
GTTTTCTTCAAAGGAAAGAGGGGAGAGTGAGGGGATAA
TGATTTAAGGTTTCGGCCGTTCATTATTTTTTTCTTCTCC
CCCAGTTATCAAGT 3'

SEQ ID NO: 19 DNA sequence for the ITS1 of *Dekkera intermedia*.

5' CAGGATGCTGGGCGCAAGCCCGTGCAGACACGTGGATA
AGTAAGGATAAAAATACATTAAATTTATTTAGTTTTAGT
CAAGAAAGAATTTTA 3'

SEQ ID NO: 20 DNA sequence for the ITS2 of *Dekkera intermedia*.

5' CCTTCTCACTATTTAGTGGTTATGAGATTACACGAGGGT
GTTTTCTTCAAAGGAAAGAGGGGAGAGTGAGGGGATAA
TGATTTAAGGTTTCGGCCGTTCATTATTTTTTCTTCTCCC
CCAGTTATCAAGT 3'

SEQ ID NO: 21 DNA sequence for the ITS1 of *Botrytis cinerea*.

5' CAGAGTTCATGCCCGAAAGGGTAGACCTCCCACCCTTG
TGTATTATTACTTTGTTGCTTTGGCGAGCTGCTTTCGGG
CCTTGTATGCTCGCCAGAGAATACCAAAACTCTTTTTAT
TAATGTCGTCTGAGTACTATATAATAGTTA 3'

SEQ ID NO: 22 DNA sequence for the ITS2 of *Botrytis cinerea*.

5' CAACCCTCAAGCTTAGCTTGGTATTGAGTCTATGTCAGT
AATGGCAGGCTCTAAAATCAGTGGCGGCGCCGCTGGGT
CCTGAACGTAGTAATATCTCTCGTTACAGGTTCTCGGTG
TGCTTCTGCCAAAACCCAAATTTTTCTATGG 3'

SEQ ID NO: 23 DNA sequence for the ITS1 of *Penicillium crustosum*.

5' CCGAGTGAGGGCCCTCTGGGTCCAACCTCCCACCCGTG
TTTATTTTACCTTGTTGCTTCGGCGGGCCCGCCTTAACT
GGCCGCCGGGGGGCTTACGCCCCCGGGCCCGCGCCCGC
CGAAGACACCCTCGAACTCTGTCTGAAGATTGAAGTCT
GAGTGAAAATATAAATTATTTA 3'

SEQ ID NO: 24 DNA sequence for the ITS2 of *Penicillium crustosum*.

5' CTGCCCTCAAGCCCGGCTTGTGTGTTGGGCCCCGTCCCC
CGATCTCCGGGGGACGGGCCCGAAAGGCAGCGGCGGCA
CCGCGTCCGGTCCTCGAGCGTATGGGGCTTTGTCACCCG
CTCTGTAGGCCCGGCCGGCGCTTGCCGATCAACCCAAA
TTTTTATCCAGG 3'

SEQ ID NO: 25 DNA sequence for the ITS1 of *Penicillium expansum*.

5' CCGAGTGAGGGCCCTTTGGGTCCAACCTCCCACCCGTGT
TTATTTACCTCGTTGCTTCGGCGGGCCCGCCTTAACTGG
CCGCCGGGGGGCTCACGCCCCCGGGCCCGCGCCCGCCG
AAGACACCCCCGAACTCTGCCTGAAGATTGTCGTCTGA
GTGAAAATATAAATTATTTA 3'

SEQ ID NO: 26 DNA sequence for the ITS2 of *Penicillium crustosum*.

5' CTGCCCTCAAGCCCGGCTTGTGTGTTGGGCCCCGTCCTC
CGATTCCGGGGGACGGGCCCGAAAGGCAGCGGCGGCAC
CGCGTCCGGTCCTCGAGCGTATGGGGCTTTGTCACCCGC
TCTGTAGGCCCGGCCGGCGCTTGCCGATCAACCCAAATT
TTTATCCAGG 3'

SEQ ID NO: 27 DNA sequence for the ITS1 of *Hanseniaspora guilliermondii*.

5' GATTGAATTATCATTGTTGCTCGAGTTCTAGTTTTAGAT
CTTTTACAATAATGTGTATCTTTATTGAAGATGTGCGCT
TAATTGCGCTGCTTTTTTAAAGTGTCGCAGTAGAAGTAA
TCTTGCTTGAATCTCAGTCAACGTTTACACACATTGGAG
TTTTTTTACTTTAATTTAATTCTTTCTGCTTTGAATCGAA
AGGTTCAAGGCAAAAAACAAACACAAACAATTTTATTT
TATTATAATTTTTTAAACTAAACCAAAATTCCTAACGGA
AATTTTAAAATAATTTA 3'

SEQ ID NO: 28 DNA sequence for the ITS2 of *Hanseniaspora guilliermondii*.

5' CCTTCTCAAAAGATAATTTTTTATTTTTTGGTTGTGGGC
GATACTCAGGGTTAGCTTGAAATTGAAGATTGTTTCAAT
CTTTTTTAATTCAACACTTAGCTTCTTTGGAGACGCTGT
TCTCGCTGTGATGTATTTATGAATTTATTCGTTTTACTTT
ACAAGGGAAATGGTAATGTACCTTAGGCAAAGGGTTGC
TTTTAATATTCATCAAGT 3'

SEQ ID NO: 29 DNA sequence for the ITS1 of *Debaryomyces carsonii*.

5' CAGTATTCTTTTTGCCAGCGCTTAACTGCGCGGCGAAAT
AAACCTTACACACAATGTTTTTTGTTATTACAGGAACTT

TTGCTTTGGCTTGTCTCTAGAAATAGAGTTGGGCCAAAG
GTTTAAACTAACTTCAATTTATTTGAACTATTTTTCTTAT
TGAAATGTCACTTTGTTGATTAAATTCAAAAAATCTTCA
3'

SEQ ID NO: 30 DNA sequence for the ITS2 of *Debaryomyces carsonii*.

5' CTCTCTCAAACCTTAGGGTTTGGTATTGAGTGATACTCT
TAGTCGAACTAGGCGTTTGCTTGAAATGTATCGGCATGA
GTGGTACTAGATTAGTGCTTCAGATTTTTCAATGTATTA
GGTTTATCCAACTCGTTGAATAGTCTGATGGCAAGTGTT
TAGTAACTATGGCTCGGCCTAACAACAACAAACAAGT
3'

SEQ ID NO: 31 DNA sequence for the ITS1 of *Pichia anomala*.

5' TAGTATTCTATTGCCAGCGCTTAATTGCGCGGCGATAAA
CCTTACACACATTGTCTAGTTTTTTTGAACTTTGCTTTGG
GTGGTGAGCCTGGCTTACTGCCCAAAGGTCTAAACACA
TTTTTTTAATGTTAAAACCTTTAACCAATAGTCATGAAA
ATTTTTAACAAAAATTAAAATCTTCA 3'

SEQ ID NO: 32 DNA sequence for the ITS2 of *Pichia anomala*.

5' CTCTCTCAAACCTTCGGGTTTGGTATTGAGTGATACTCT
GTCAAGGGTTAACTTGAAATATTGACTTAGCAAGAGTG
TACTAATAAGCAGTCTTTCTGAAATAATGTATTAGGTTC
TTCCAACTCGTTATATCAGCTAGGCAGGTTTAGAAGTAT
TTTAGGCTCGGCTTAACAACAATAAACTAAAAGT 3'

SEQ ID NO: 33 DNA sequence for the ITS1 of *Pichia kluyveri*.

5' CTGTGATTTATATCTTATACACATGCGTGAGCGCACCAA
ACACCTAAAATTGTAATAATACCAGTCACTAAGTTTTAA
CAAAACA 3'

SEQ ID NO: 34 DNA sequence for the ITS2 of *Pichia kluyveri*.

5' CCTTCTTGCGCAAGCAGAGTTGAGAACAGGCTATGCCTT
TTTCGAAATGGAACGTCGTGGACGAAGTGAACTAAACT
TTTAGCACGCTTTGGCCGCCGAACTTTTAACTAAGC 3'

SEQ ID NO: 35 DNA sequence for the ITS1 of *Candida krusei*.

5' CTGTGATTTACTACTACACTGCGTGAGCGGAACGAAAA
CAACAACACCTAAAATGTGGAATATAGCATATAGTCGA
CAAGAGAAATCTACGAAAAAACAAACA 3'

SEQ ID NO: 36 DNA sequence for the ITS2 of *Candida krusei*.

5' CCATCTTGCGCGTGCGCAGAGTTGGGGGAGCGGAGCGG
ACGACGTGTAAAGAGCGTCGGAGCTGCGACTCGCCTGA
AAGGGAGCGAAGCTGGCCGAGCGAACTAGACTTTTTTT
CAGGGACGCTTGGCGGCCGAGAGCGAGTGTTGCGAGAC
AACAAAAAGC 3'

SEQ ID NO: 37 Oligonucleotide Sequence SXUITS1a.

5' TTTTGGCAAGAGCATGAGAGCTTTTACTGGGCAAGAAG
ACAAGAGATGGAGAGTCCACCC 3'

SEQ ID NO: 38 Oligonucleotide Sequence SXLITS2a.

5' CCGTACTTGCATTATACCTCAAGCACGCAGAGAAACCT
CTCTTTGGAAAAAAAAACA 3'

SEQ ID NO: 39 Oligonucleotide Sequence SXUITS1b.

5' TGGCAAGAGCATGAGAGCTTTTACTGGGCAAGAAGACA
AGAGATGGAGAGTCCACCCGGG 3'

SEQ ID NO: 40 Oligonucleotide Sequence SXLITS2b.

5' CGACCGTACTTGCATTATACCTCAAGCACGCAGAGAAA
CCTCTCTTTGGAAAAAAAAAC 3'

SEQ ID NO: 41 Oligonucleotide Sequence SLUITS1a.

5′ CTGTTTATAAACAGACGGTAGACTTTTCTTGGGGAGAGG
TTGTTGATTGCTTTGGCCTGCGCTTAACTGCGCGGCTAG
TAGTTGATGATTTTGTTATTATCCGAGACGAA 3′

SEQ ID NO: 42 Oligonucleotide Sequence SLLITS2a.

5′ TAATAAAAACTCTCAATAATCACTAGTTGGGATAAACC
TAATAGTAATAAACAAGAAATT 3'

SEQ ID NO: 43 Oligonucleotide Sequence SLUITS1b.

5′ TTTATAAACAGACGGTAGACTTTTCTTGGGGAGAGGTTG
TTGATTGCTTTGGCCTG 3′

SEQ ID NO: 44 Oligonucleotide Sequence SLLITS2b.

5′ CTGTAATAAAAACTCTCAATAATCACTAGTTGGGATAA
ACCTAATAGTAATAAACAAGAAAT 3′

SEQ ID NO: 45 Oligonucleotide Sequence BRUITS1a

5′ GGTGAACCTGCGGAAGGATCATTACAGGATGCTGGGCG
CAAGCCCGTGCAGACACGTGGATAAGCAAGGATAAAAA
TACAT 3′

SEQ ID NO: 46 Oligonucleotide Sequence BRLITS2a.

5' ACTGGGGGAGAAGAAAAAAATAATGAACGGCCGAAAC
CTTAAATCATTATCCCCTCACTCTCCCCTCTTTCCTTTGA
AGAAAACACCCTCGTGTAATCTCATAACCACTA 3′

SEQ ID NO: 47 Oligonucleotide Sequence BRUITS1b.

5′ GGTGAACCTGCGGAAGGATCATTACAGGATGCTGGGCG
CAAGCCCGTGCAGACACG 3′

SEQ ID NO: 48 Oligonucleotide Sequence BRLITS2b.

5' AACTGGGGGAGAAGAAAAAATAATGAACGGCCGAAAC
CTTAAATCATTATCCCCTCACTCTCCCCTCTTTCCTTTGA
AGAAAACACCCTCGTGTAATCTCATAACCACTA 3'

SEQ ID NO: 49 Oligonucleotide Sequence BCUITS1a.

5' TGGCGAGCTGCTTTCGGGCCTTGTATGCTCGCCAGAGAA
TACCAAAACTCTTTTTAT 3'

SEQ ID NO: 50 Oligonucleotide Sequence BCLITS2.

5' GCGCCGCCACTGATTTTAGAGCCTGCCATTACTGACATA
GACTCAATACCAAGCTAA 3'

SEQ ID NO: 51 Oligonucleotide Sequence BCUITS1b.

5' GCTGCTTTCGGGCCTTGTATGCTCGCCAGAGAATACCAA
AACTCTTTTTATTAATGT 3'

SEQ ID NO: 52 Oligonucleotide Sequence PXUITS1a.

5' CCCTYTGGGTCCAACCTCCCACCCGTCTTTATTTTACCT
TGTTGCTTCGGCGGGCCCGCC 3'

SEQ ID NO: 53 Oligonucleotide Sequence PXLITS2a.

5' TCGGCAAGCGCCGGCCGGGCCTACAGAGCGGGTGACAA
AGCCCCATACGCTCGAGGACCGGACGCGGTGC 3'

SEQ ID NO: 54 Oligonucleotide Sequence PXUITS1b.

5' CCCTYTGGGTCCAACCTCCCACCCGTCTTTATTTACCTC
GTTGCTTCGGCGGGCCCGC 3'

SEQ ID NO: 55 Oligonucleotide Sequence PXLITS2b.

5′ AGCGCCGGCCGGGCCTACAGAGCGGGTGACAAAGCCCC ATACGCTCGAGGACCGG 3′

SEQ ID NO: 56 Oligonucleotide Sequence HGUITS1.

5′ AATCTTGCTTGAATCTCAGTCAACGTTTACACACATTGG AGTTTTTTTACTTTAATTTAATTCTTTCTGCTTTGAATCG AAAGGTTCAAGGCAAAAAAC 3′

SEQ ID NO: 57 Oligonucleotide Sequence HGLITS2.

5' TAAATACATCACAGCGAGAACAGCGTCTCCAAAGAAGC TAAGTGTTGAATTAAAAAAGATT 3′

SEQ ID NO: 58 Oligonucleotide Sequence DXLITS2a.

5′ CTCATGCCGATACATTTCAAGCAAACGCCTAGTTCGACT AAGAGTATCACTCAATACCAAA 3′

SEQ ID NO: 59 Oligonucleotide Sequence DXLITS2b.

5′ TAAACACTTGCCATCAGACTATTCAACGAGTTGGATAA ACCTAATACATTGAAAAATCTGA 3′

SEQ ID NO: 60 Oligonucleotide Sequence PAUITS1.

5' TTGCTTTGGGTGGTGAGCCTGGCTTACTGCCCAAAGGTC TAAACACATTTTTTTAATGT 3′

SEQ ID NO: 61 Oligonucleotide Sequence PALITS2.

5′ ATACATTATTTCAGAAAGACTGCTTATTAGTACACTCTT GCTAAGTCAATATTTCAAGTTA 3'

SEQ ID NO: 62 Oligonucleotide Sequence PKLITS2.

5′ GCGGCCAAAGCGTGCTAAAAGTTTAGTTCACTTCGTCCA
CGACGTTCCATTTCGAAAAAGG 3′

SEQ ID NO: 63 Oligonucleotide Sequence CKUITS1.

5′ GCGGAACGAAAACAACAACACCTAAAATGTGGAATATA
GCATATAGTCGACAAGAGAAATC 3′

SEQ ID NO: 64 Oligonucleotide Sequence CKLITS2.

5′ TCAGGCGAGTCGCAGCTCCGACGCTCTTTACACGTCGTC
CGCTCCGCTCCCCCAACTCT 3'

SEQ ID NO: 65 Oligonucleotide Sequence SXU99.

5′ CTTTTACTGGGCAAGAAGAC 3′

SEQ ID NO: 66 Oligonucleotide Sequence SXL658.

5′ AAGCACGCAGAGAAACC 3′

SEQ ID NO: 67 Oligonucleotide Sequence SXU102.

5′ TTACTGGGCAAGAAGACAAG 3′

SEQ ID NO: 68 Oligonucleotide Sequence SXL661.

5' CTCAAGCACGCAGAGAA 3′

SEQ ID NO: 69 Oligonucleotide Sequence SLU85.

5′ GACTTTTCTTGGGGAGAG 3′

SEQ ID NO: 70 Oligonucleotide Sequence SLL635.

5' TCACTAGTTGGGATAAACCT 3'

SEQ ID NO: 71 Oligonucleotide Sequence SLU88.

5' TTTTCTTGGGGAGAGG 3'

SEQ ID NO: 72 Oligonucleotide Sequence SLL636.

5' ATCACTAGTTGGGATAAACC 3'

SEQ ID NO: 73 Oligonucleotide Sequence SLU136.

5' CGGCTAGTAGTTGATGATT 3'

SEQ ID NO: 74 Oligonucleotide Sequence SLL634.

5' CACTAGTTGGGATAAACCTA 3'

SEQ ID NO: 75 Oligonucleotide Sequence BRU53A.

5' ATTACAGGATGCTGGGC 3'

SEQ ID NO: 76 Oligonucleotide Sequence BRU53B.

5' ATTACAGGATGCTGGG 3'

SEQ ID NO: 77 Oligonucleotide Sequence BRU77.

5' CGTGCAGACACGTGGAT 3'

SEQ ID NO: 78 Oligonucleotide Sequence BRL339.

5' CTTTGAAGAAAACACCCTC 3'

SEQ ID NO: 79 Oligonucleotide Sequence BRL367.

5′ AATCATTATCCCCTCACTC 3′

SEQ ID NO: 80 Oligonucleotide Sequence BRL390.

5′ TAATGAACGGCCGAAAC 3′

SEQ ID NO: 81 Oligonucleotide Sequence BCU136.

5′ TTGTATGCTCGCCAGAG 3′

SEQ ID NO: 82 Oligonucleotide Sequence BCL393.

5′ GCCTGCCATTACTGACA 3′

SEQ ID NO: 83 Oligonucleotide Sequence BCU142.

5′ GCTCGCCAGAGAATACC 3′

SEQ ID NO: 84 Oligonucleotide Sequence PXU87.

5′ ACCCGTCTTTATTTTACCTT 3′

SEQ ID NO: 85 Oligonucleotide Sequence PXL495.

5′ CTACAGAGCGGGTGACA 3′

SEQ ID NO: 86 Oligonucleotide Sequence PXU86.

5′ CACCCGTGTTTATTTTACCT 3′

SEQ ID NO: 87 Oligonucleotide Sequence PXL482.

5′ CAAAGCCCCATACGC 3′

SEQ ID NO: 88 Oligonucleotide Sequence PXL491.

5′ AGCGGGTGACAAAGC 3′

SEQ ID NO: 89 Oligonucleotide Sequence HGU193.

5′ CAACGTTTACACACATTGG 3′

SEQ ID NO: 90 Oligonucleotide Sequence HGU231.

5′ AATTCTTTCTGCTTTGAATCG 3′

SEQ ID NO: 91 Oligonucleotide Sequence HGL601.

5′ CAGCGTCTCCAAAGAAGCTAA 3′

SEQ ID NO: 92 Oligonucleotide Sequence DXL447.

5′ GCAAACGCCTAGTTCGACTAA 3′

SEQ ID NO: 93 Oligonucleotide Sequence DXL526.

5′ ATTCAACGAGTTGGATAAACC 3′

SEQ ID NO: 94 Oligonucleotide Sequence PAU133.

5′ GGCTTACTGCCCAAAGGTC 3′

SEQ ID NO: 95 Oligonucleotide Sequence PAL451.

5′ TGCTTATTAGTACACTCTTGC 3′

SEQ ID NO: 96 Oligonucleotide Sequence PKL356.

5′ GTTTAGTTCACTTCGTCCACG 3′

SEQ ID NO: 97 Oligonucleotide Sequence CKU104.

5′ CCTAAAATGTGGAATATAGCA 3′

SEQ ID NO: 98 Oligonucleotide Sequence CKL354.

5′ ACGCTCTTTACACGTCGTC 3′

SEQ ID NO: 99 Oligonucleotide Sequence ITS5.

5′ GGAAGTAAAAGTCGTAACAAGG 3′

SEQ ID NO: 100 Oligonucleotide Sequence ITS4.

5′ TCCTCCGCTTATTGATATGC 3′